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16. ABSTRACT

This paper reviews major categories of pathobiological research, citing strengths and weaknesses of each. Where appropriate, examples from the aquatic toxicology literature are cited. Finally, the application of the pathobiological approach to field studies and the importance of findings in aquatic species to other species including man are discussed.

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Pathobiological Measures of Marine Pollution Effects

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INTRODUCTION

The class of techniques embodied in the term "pathobiology" includes structure/function analyses of organs, tissues, cells and subcellular components (organelles, cytosol, enzymes and other macromolecules) derived--in experimental toxicology--from control and diseased* organisms. Since the cell is the functional unit of all plant and animal organization, pathobiologic mechanisms apply to all phyletic systems. Rationale and methodology of pathobiology are found in extensive reviews (Forbus 1952; Majno et al. 1960; Hill 1975; Trump and Arstila 1975; Scarpelli and Trump 1971). A few basic principles are particularly relevant in this workshop. Disease is not associated with new, different structure and function; rather, quantitative alterations (increase or decrease) of existing structure and function occur (Forbus 1952). To understand disease, we must understand alterations in structure/function occurring in response to various injuries. Although the number of etiologic agents

*In this paper, disease includes all reactions to injury associated with pollutants in the aquatic environment and may incorporate infectious disease.

may include many pollutants and pathogenic organisms, we can be encouraged by the fact that a long list of etiologic agents of disease does not reflect an equally long list of pathogenetic mechanisms (Hill 1975). An organism's genetic composition is of importance relative to initial susceptibility to injury; however, the manifestation of disease reflects disturbances in enzymatic activity. Since enzymes are associated with specific organelles of cells, altered enzyme activity is often reflected as structural alteration (Hill 1975; Trump and Arstila 1975; Scarpelli and Trump 1971) underscoring the need for correlated structure/function studies. Since experimental procedures in pathobiology include morphological, biochemical and physiological tests, it is impossible in one review to cite all work encompassed by this term. Rather, the intent is to review major categories of pathobiological research and to present strengths and weaknesses of each. Where appropriate, examples from the aquatic toxicology literature are cited. Finally, the application of the pathobiologic approach to field studies and the importance of findings in aquatic species to other species including man are discussed.

GENERAL METHODOLOGY OF PATHOBIOLOGICAL ASSESSMENT

Table 1 presents some of the laboratory pathobiological tests used in determining pollutant effect. Hypotheses, assumptions, controls and the type data are given for each test with some information on statistical evaluation.

Morphologic Examination

This includes gross inspection of fresh or fixed individuals to yield information on weight, size, shape, number, coloration and surface texture. Microscopic inspection uses magnification to display structures in greater detail. The underlying assumption of morphological approaches in toxicity studies is that the investigator can distinguish between "normal" and altered structure and, among the latter, premortem (i.e., toxicant induced) from postmortem (autolytic) change. Although all fixed tissues have been removed from their blood supply and can be considered irreversibly injured, the morphologic analysis of adequately fixed material may reveal the following general populations of cells: those not altered by toxicant prior to somatic death and maintained in a normal, nonaltered state; those altered but not killed prior to somatic death, in which certain features associated with sublethal injury (swelling, fat vacuolation, or accumulation of stainable cytoplasmic inclusions) would be seen (Trump and Arstila 1975); and those altered lethally (before somatic death), in which autolysis (necrosis) would have occurred (Scarpelli and Trump 1971) prior to fixa-

Table 1. Considerations for validity of experimental design in laboratory pathobiological tests.

Test	Hypothesis[a]	Assumptions	Controls	Data/Evaluation
Routine morphologic	Exposure results in structural alteration at cell, tissue, organ or organism level.	Normal structure known at all levels of organization.	Paired for sex, age, size; all conditions same except no toxicant; region and orientation of tissue identical in exposed and control animals.	Target organ determination; degree injury established; determine number affected at each toxicant concentration (chi-square); determine % incidence of lesion in each experimental group.
Morphometric	Exposure results in quantifiable response at cell, tissue, or organ level.	Structural entities recognizable at each level of organization; 3-dimensional information can be obtained from 2-dimensional sections.	See controls for routine morphologic test; randomization must be ensured.	Volume compartment; surface density; numerical density and length of tissue components in reference volume of cell or tissue. Data are numerical and are amenable to statistical evaluation.

[a] As used here is overall (not statistical) hypothesis.

Table 1. Continued

Test	Hypothesis[a]	Assumptions	Controls	Data/Evaluation
Marker enzyme activity in cells of target tissues of organism exposed to toxicant	Exposure results in altered enzyme activity of specific cellular organelles.	Recovery of specific subcellular fractions identical in control and treated animals.	Control conditions of the routine morphologic test must be observed. Estimates of recovery of marker enzyme activity in treated and control population essential.	Data (numerical) are amenable to statistical evaluation.
Serum enzyme activity in organisms exposed to toxicant	Exposure results in change in serum enzyme (or isozyme) levels.	Damaged cell populations release tissue-specific recoverable in serum.	See controls of routine morphologic test. Same conditions for enzyme assays in sera from both.	Data are amenable to statistical evaluation.
Other fluids in organism	Elevation or depression of specific molecular entities (e.g., ions, sugars, fats, proteins) follows exposure.	Toxicant-induced dysfunction results in imbalance.	See controls of routine morphologic and serum enzyme tests.	Data are amenable to statistical evaluation.

Staining (histochemistry)	Exposure results in appearance or loss of reaction product of enzyme or cellular component (glycogen, fat, mucus) in affected cell populations.	Quantitative difference will be detectable.	See controls of routine morphologic test plus biologic and "no substrate".	Presence or absence of change scored per individual; strength of reaction graded semiquantitatively (+1, +2, etc.) using well-defined criteria. If reaction stoichiometric, can quantify using scanning microdensitometer. If areal analysis of affected foci conducted, statistics can be applied (Volume affected area/ Volume organ).

[a] As used here is overall (not statistical) hypothesis.

tion. In addition, certain cells which responded to chemotactic factors released by dead and dying cells (Ward 1975) in the living organism may be found as accumulations in tissue sites not normally the loci for these cells.

The scope of this conference does not permit detailed description of techniques used in the morphologic phase of pathobiologic studies. Therefore, only brief mention will be made of procedures essential for validity of morphologic examination. Suitable procedures of necropsy, fixation, dissection/trimming, processing and light microscopic analysis of aquatic animals including zooplankton, sponges, hydroids, anemones, corals, worms, barnacles, bivalves, gastropods, cephalopods, crustaceans, echinoderms and fish have been compiled (Galigher and Kozloff 1971; Couch et al. 1974; Yevich and Barszcz 1982; Hinton et al. 1973). Fixatives suitable for both routine light and electron microscopy are available (Ito and Karnovsky 1968; McDowell and Trump 1976) and use of these is recommended unless decalcification is considered essential, in which case use of alternative fixatives may be necessary (Yevich and Barszcz 1982). In addition to fixation, orientation of organs prior to embedment in paraffin or plastic is important to ensure comparison between identical regions of organs and tissues in control and exposed animals. Distinguishing premortem from postmortem change is also important. Studies of controlled in vitro autolysis (Majno et al. 1960; Trump and Arstila 1975; Trump et al. 1971) have shown that lethally injured cells undergo a recognizable series of morphologic changes during the necrotic phase (degradative reactions occurring after cell death--collectively termed autolysis (Trump and Arstila 1975)). Direct chemical fixation of cells causes death without necrosis (Trump and Arstila 1975). However, in the case of improperly fixed tissue, distinguishing lethally injured cells produced by toxicant from those which underwent autolysis between necropsy (at which time tissue was removed from blood supply) and actual time of chemical fixation, becomes difficult. Data on controlled in vitro autolysis include invertebrates (Sparks 1972) and fishes (unpublished data of Gardner discussed in Couch et al. 1974). An understanding of these processes in tissues of test organisms helps achieve meaningful morphologic data.

By means of a routine light microscopic morphologic approach, the target organ of a given pollutant can be determined. Specific regions of tissue response within the target organ can also be established. When marine shrimp Penaeus duorarum were exposed to cadmium dissolved in seawater, gross and light microscopic examination revealed gill alteration which appeared as blackened regions (Couch 1977). More detailed analysis revealed foci of alteration primarily in terminal filaments. When analysis by electron microscopy was performed, organelle alteration including nuclear lysis, mitochondrial swelling and myelin-line membrane accumulation

was consistent with cellular necrosis. Hawkes (1980) reviewed literature on effects of xenobiotics on marine fish tissues after exposure to chlorobiphenyls or crude oil. Morphologic analysis after laboratory exposure shows olfactory epithelium as a target tissue in Menidia menidia exposed to crude oil. Other sites showing structural evidence of toxicity after exposure to crude oil included lens and intestine. When juvenile Chinook salmon, Oncorhynchus tshawytscha, were fed a diet containing a model mixture of petroleum hydrocarbons, chlorinated biphenyls or both (Hawkes et al. 1980), cellular change, primarily in columnar cells of intestinal mucosa, indicated toxicity in this organ. When the estuarine fish, the sheepshead minnow, Cyprinodon variegatus, was exposed to Kepone, an organochlorine, scoliosis resulted (Couch et al. 1977). In addition, light microscopic and x-ray analysis revealed breaking of centra of vertebrae at the epicenter of the spinal flexure.

All the above relate to laboratory exposures of organisms to specific pollutants. Sindermann (1979) reviewed lesions which have been found in feral marine organisms after gross and microscopic examination. These included fin erosion, ulcers, shell disease of crustacea, lymphocystis, stress provoked latent infections, neoplasms, skeletal anomalies and genetic abnormalities.

Data derived from routine morphologic analysis are valuable in determining which experimental probes should follow (i.e., ultrastructural study, analysis of organ homogenates or analysis of cellular and/or subcellular fractions from specific cell types after their separation). If the number of affected individuals can be determined and expressed for each toxicant concentration, data are amenable to statistical evaluation by tests such as the chi-square. Another application of a routine morphologic approach is in chemical carcinogenesis research using teleost species, where the end point, a clearly defined structural entity with specific characteristics, is usually detected by routine light microscopic techniques and the incidence data for tumors in each experimental group are listed (Ishikawa et al. 1975; Aoki and Matsudairo 1977; Pliss and Khudoley 1975; Hendricks et al. 1980 a,b). The routine morphologic approach is of additional importance when one considers that it provides a relatively quick initial screen to gain information from a variety of organ systems simultaneously.

Morphometry

Since the overall objective of the pathobiologic approach to toxicity studies is correlation of altered structure and function, it becomes necessary to employ tests which may provide for integration of morphological, biochemical and physiological studies. The non-quantitative appearance of morphologic data (i.e., micrographs) has seriously hampered full integration of studies incorporating cellular fractionation on one hand and electron microscopy on the other (Reide and Reith 1980). However, methods to extract

numerical data from microscopic images have evolved (Reide and Reith 1980; Bolender 1980, 1981; Elias et al. 1971; DeHoff and Rhines 1968; Underwood 1970; Weibel and Bolander 1973; Weibel 1979, 1980). Based upon fundamental principles defined in mathematical terms (Elias et al. 1971; Weibel 1979, 1980), morphometry permits the quantitative study of tissue and cell structures. Coupled with computer assisted technology, quantitative work can be performed in reasonable time (Rohr et al. 1976). Thus, morphometry is regarded as a necessary element in interdisciplinary studies such as toxicology which involve morphologists, biochemists, physiologists or pharmacologists (Bolender 1980). By using morphometry, the volume, surface area and number of structural features can be objectively and reproducibly determined.

For the determination of volume, a point network (lattice) with total points = P_t is superimposed over the micrograph or the projected image of the glass slide and point counts within a given profile (P_i) are determined. This requires a judgment call by the observer at each point and is recorded for each. The volume density of the structure i (V_{vi}) is proportional to P_i/P_t which is proportional to P_{Pi}. The total number of test points that must be applied to one representative sample unit depends on V_v and on the relative error considered acceptable. Formulae (Weibel 1979) exist for determining the number of test points needed per representative sample. In practice, one roughly estimates the order of magnitude of V_v on a few micrographs and then decides upon the total points (P_t). Table 2 illustrates the multistage morphometric analysis. At stage 1, the weight and volume of the organ or possibly organism (some invertebrates) is determined. The second stage of investigation, using light microscopic observation of fixed material, is conducted at the organ/tissue level of organization. At this level, for example, points lying over parenchyma, stroma, blood vessels and ducts would be determined. The subsequent stage of examination, at the level of individual cells, requries high resolution light microscopy (Epon or methacrylate sections) or, more commonly, low magnification electron microscopy. Analysis of these sections determines the total number of points lying over nuclei and cytoplasm of all individual cell types. In addition, the total number of points over extracellular space is determined. Linear intercepts with structures such as nuclear and plasma membranes are quantified to determine surface density of each. The fourth level of organization involves subcellular structures (organelles). Here the total number of points lying over specific organelles and the linear intercepts with membranous structures are determined to derive the volume density and surface area of structures. This level of investigation is done under medium to high magnification using the electron microscope. Procedures for morphometry require that randomization be carried out at the animal level, at the level of tissue blocks, and within areas of sectioned and stained material. Collecting tissues for morphometric examination can follow gener-

Table 2. The multistage morphometric analysis[a].

Stage	Level of Structural Organization	Data
1	Organ or organism	Weight, volume
2	Organ/Tissue	P_i parenchyma, stroma, blood vessels, ducts, etc.
3	Cell	P_i nuclei and cytoplasm of all individual cell types P_i extracellular space P_i mitochondria I_1 nuclei
4	Organelle	P_i mitochondria I_i endoplasmic reticulum

[a] For complete details see Rohr et al. (1976)

P_i = total points over tissue component

I_1 = linear intercepts with structure

al procedures used in routine morphologic examination. In addition, the investigator, using the multistage morphometric analysis (Table 2) controls the final level of organization from which information is sought. It may not be necessary in all instances to proceed to the electron microscope. Once the material has been collected for such analysis, the option is there, should such investigation prove necessary.

Hughes (1972) applied morphometric examination in studies of gas exchange regions of gill in tench, trout, dogfish, icefish and catfish. Exp sing gill area per body weight, he found no good correlation a g species. When distribution of gill area was analyzed, filament lengths varied as a function of their number. Shape

of secondary lamellae differed at various sites along filaments. On the basis of the above findings, Hughes recommended analysis of the following parameters in gill toxicity studies: relative proportion of specific cell types and extracellular material in secondary lamellae; surface area of blood channels in secondary lamellae; and effect of pollutants on different parts of the water/blood barrier. In a subsequent study, Hughes et al. (1979) used morphometric techniques to determine O_2 diffusion capacities in gills of control, nickel-, chromium- and cadmium-treated trout. Based on measurements of blood/water barrier, nickel exposure produced a marked harmful effect in diffusion capacity. Recovery to normal values was seen in trout 21 days after exposure. These workers concluded that morphometric methods can be combined with physiological data to have a quantitative, and possibly a predictive value, for assessing the effects of pollution on gills and on other organ systems.

Zuchelkowski et al. (1980) studied the effects of acid water (H_2SO_4 addition, pH 5.7-6.15) on alcian blue-periodic acid Schiff's stainable mucosubstances of epidermis in brown bullhead catfish, Ictalurus nebulosus. Standard morphometric techniques were used to determine mucous cell volume density and number density. Mucous cell volume density was significantly increased in fish exposed to acid stress for 5 days. Since the number of mucous cells was nearly twice that of controls while the volume of individual cells was the same, these workers concluded that acid stress results in increased number (hyperplasia) but not volume of individual mucous cells.

Subsequently, Zuchelkowski and Lantz (1981) reported sexual dimorphism in brown bullhead skin with respect to mucous cells. Control female bullheads had more mucous cells per volume of epidermis than did males. After acid stress, the sex differences were not seen. If male fishes predominate in assay populations, greater responses of mucous cells to acid stress will be encountered (Zuchelkowski and Lantz 1981).

Sex associated differences in epidermis may be due to hormonal levels in individual fish. Schwerdtfeger (1979a,b) used morphometric methodology to quantitatively examine epidermis of the guppy, Poecilia reticulata, following treatment with prolactin, thyroxine, and testosterone. When freshwater adapted guppies were treated with prolactin, morphologic parameters in epidermis which were increased included number of mucous cells, number of cell layers and the number of subepidermal capillaries. Upon adaptation to seawater (Schwerdtfeger 1979a), epidermis of guppies showed increased surface extent of superficial cells, an increase in the number of small, electron-dense vesicles and increased occurrence of chloride cells. Thyroxine in low doses caused an increase in number of mucous cells and small, electron-dense vesicles as well as increased height of glycocalyx (Schwerdtfeger 1979b).

In addition to the above, morphometry has been applied to analysis of changes during carcinogenesis in liver of Japanese medaka, Oryzias latipes (Hinton et al. 1982a).

Marker Enzyme Tests

This third category of laboratory pathobiological tests (Table 1) measures "marker" enzyme activity in cells of target tissues. Before this type of biochemical data can be collected, an organ must be homogenized and fractionated. Unfortunately, spatial relationships of organelles in a single cell and/or organelles from several different types of cells are lost (Bolender 1980). The strength of the biochemical approach resides in the accuracy with which an enzyme activity can be measured in a given fraction. Within the framework of analytical fractionation, the amount of activity in the various fractions can be compared with that in the original homogenate by calculating recoveries (DeDuve and Berthet 1954). Subcellular fractionation of rainbow trout liver homogenates has been studied to assess homogeneity of fractions (Statham et al. 1977), and a refined fractionation protocol has been developed. While such methods can be used to identify membrane marker heterogeneities, the initial homogenization procedure resulted in a mixture of membranes from a variety of cell types. Therefore, the cellular origin of the heterogeneities remains obscured (Bolender 1980). If we consider teleost liver in general, abundant morphologic heterogeneity is present, and the following cell types are seen: hepatocytes, endothelial cells, Kupffer cells, fat-storing cells, bile ductular epithelial cells, fibroblasts, macrophages, circulating blood cells and exocrine pancreatic cells (Hinton and Pool 1976). From this the need for "coupled" morphologic and biochemical assays (Bolender 1980) is readily apparent.

Organ Dysfunction Tests

These may be grouped into four broad categories. The first includes measurement of substances circulating in the blood, which depend upon a specific organ for their production or removal. The second group of tests assays substances in the serum which indicate abnormal function. In the third, plasma disappearance rates are determined using dyes and other substances which, following administration, are removed by a specific organ. Last, tests can be administered to determine the capacity of an organ to synthesize a given product when an essential precursor is furnished. Organ dysfunction tests are reviewed in standard texts on clinical laboratory diagnosis (Davidsohn and Henry 1974) or in monographs on specific organs such as the liver (Levy 1965). Some substances released from damaged cells are useful in the diagnosis of cell damage. Detectable in very low concentrations, enzymes reach a peak in the plasma following release from damaged cells. All cells have similar intracellular enzymes with similar organelle localization. How-

ever, in the human, the use of organ-specific isoenzymes (Zimmerman and Henry 1974) has greatly enhanced identification of the injured organ. Although isoenzymes of phosphoglucomutase, isocitrate dehydrogenase, and glucose-6-phosphate dehydrogenase specific for teleost lever and muscle are known to exist (Allendorf et al. 1977), little use in toxicologic assays has been forthcoming.

In an effort to determine the response of rainbow trout (Salmo gairdneri) liver to a proven hepatotoxic compound, carbon tetrachloride (CCl_4), studies were performed using serum enzymes (Statham et al. 1978; Pfeifer et al. 1980) and the dye, sulfobromophthalein (BSP) (Gingerich et al. 1978), which is selectively cleared from plasma by the liver and excreted in the bile. Exposure caused elevation of serum enzymes (Statham et al. 1978; Pfeifer et al. 1980) and induced retention of BSP (Gingerich et al. 1978). Chemical assay of liver revealed ^{14}C residue of CCl_4 in hepatic homogenate and showed increased diene conjugation indicating membrane toxicity (Statham et al. 1978). Recently, Gingerich (1982) reviewed literature on hepatic toxicology and covered the plasma enzymes and clearance studies in detail.

Urine, another body fluid available for assay (Table 1), has been studied in trout before and after CCl_4 exposure (Pfeifer and Weber 1980). Decreased urine flow, increased urine osmolality and proteinuria characterized trout exposed to CCl_4.

In a study of the effects of chlorine produced oxidants (CPO) on juveniles of the marine fish, Leiostomus xanthurus, Middaugh et al. (1980) used analysis of blood pH, oxygen uptake and gill morphology to establish toxicity. At sublethal concentrations of CPO, blood pH was decreased, oxygen uptake was depressed and gill morphology was altered. The latter included lesions of oxygen exchange portions of the gill and included sloughing of respiratory epithelium from underlying vasculature. These workers felt that the gill alterations could easily account for the change in blood pH and oxygen uptake.

Although relatively little application of serum and other body fluid assays is evident in aquatic toxocologic investigations, the above studies clearly indicate their merit. It should be noted that careful consideration of microchemical assays will have to be given when specimens of species with small body size are used.

Staining (Histochemistry)

This technique (see Table 1) represents a powerful tool for localization of enzymes and other cellular components (secretion products, hormones, lipids, proteins and carbohydrates) within tissues and, when applied at the cytochemical level, within cells. Although not directly quantifiable, except in instances where stoichiometric reaction can be demonstrated, the techniques provide a method for in situ characterization of sites of enzyme alteration. Studies of mucosubstances in skin and intestinal tract of teleosts

commonly employ these techniques (Reifel and Travill 1979; Zuchelkowski et al. 1980; Hinton et al. 1982b). Although as yet rarely employed in aquatic carcinogenesis studies (Scarpelli et al. 1963), enzyme histochemistry has been a valuable tool in characterization of small foci and other putative precursor lesions for epithelial tumors in mammalian carcinogen bioassay (Kalengayi and Desmet 1975; Lipsky et al. 1981).

Structure/Function Correlation

As was stated in the introduction, this is the goal of pathobiologic investigation. This objective, pursued in vitro using teleost kidney preparations (Forster 1948; Trump and Bulger 1971; Trump et al. 1975; Trump and Jones 1977), involved a variety of inhibitors and conditions to definitively modify functional parameters (transport of dye). Identical preparations, functionally defined, were then processed for ultrastructural analysis. In these correlated studies membranes of various organelles, especially plasma and mitochondrial membranes, revealed alterations concomitant with reduced transport function. A pattern of morphologic reaction is emerging in transporting epithelium which can be linked to functional impairment (Pritchard and Miller 1980).

The studies by Hughes et al. (1979) are particularly encouraging. Gill structure/function alteration was produced by laboratory exposure, and, under conditions of recovery, values were obtained which indicated a return to the control state.

One response, common across phyla including both aquatic and terrestrial organisms, has been the morphologic alteration of hepatic and/or hepatopancreatic endoplasmic reticulum after exposure to certain xenobiotics, specifically petroleum hydrocarbons and chlorobiphenyls (Couch and Nimmo 1974; Sabo and Stegeman 1977; Hinton et al. 1978; Lipsky et al. 1978; Klaunig et al. 1978; Schoor and Couch 1979; Hawkes 1980). Structure/function correlation has been shown in biochemical and coupled morphologic (quantitative) studies of rat liver endoplasmic reticulum after phenobarbital exposure (Bolender 1980). In fish exposed to polychlorinated biphenyls or aflatoxin (Klaunig et al. 1978; Scarpelli 1976) biochemical induction correlated with an apparent increase in endoplasmic reticulum. Clearly morphometric analysis of this alteration is needed to correlate with functional data.

Bolender (1980) emphasizes the necessity for a common unit of reference if correlation between biochemical and morphologic data are to be obtained. Where some workers failed to demonstrate correlation of morphologic and chemical (serum enzyme) data, a lack of common reference unit is apparent. For example, light microscopic subjective morphologic evaluation of liver (Pfeifer et al. 1980) and of kidney (Pfeifer and Weber 1980) toxicity following CCl_4 failed to show correlation with elevated serum enzyme levels and urine parameters respectively. Both serum enzyme elevations

and proteinuria imply cellular alteration, yet routine histologic techniques, which yield little if any information at the cellular level were used in the companion morphologic studies. For a common unit of reference to exist, morphologic probes at the cellular and/or subcellular level would be necessary and morphometric studies would be needed to provide objective numerical data for comparison.

Application of Pathobiologic Tests to Field Studies

Provided a power source and equipment are available for fractionation, freezing, and fixation of tissues, no apparently insurmountable difficulty exists in on-site collection of field samples for subsequent laboratory pathobiologic examination. It is clear that the tests can be performed in a reproducible manner, are scientifically valid and, when administered properly, yield data amenable to statistical evaluation. The central problem in the application of pathobiologic as well as other forms of tests to field studies is illustrated in Figure 1 which was modified from a figure in the elegant paper by Snieszko (1974) which describes many of these interactions in detail. Conditions affecting the field test can change with respect to the animal (host), the environment and the pathogenic agent(s). A partial listing of host factors (circle at top left) includes age, sex, genetic strain, nutritional status and the history of prior exposure to xenobiotics which might influence the rate of metabolism of potentially pathogenic chemicals (Lech and Bend 1980). Environment is represented in the bottom circle of Figure 1. Environmental factors and the physiologic adaptations of fishes have been the subject of an in depth and recent review (Love 1980). In that review, specific factors in addition to those given in Figure 1 include stress other than crowding; geographical influence; and depth, including pressure and illumination. The overlapping of the circles (host and environment) in Figure 1 signifies interaction. Since even minor variations in the external environment require adjustment by the cell or organism, any major variations of course require major adjustments. The inherent dynamic nature of the environment and the host adds additional complexity to experimental design since factors may change acutely or remain altered for some time (chronic interaction). The circle at the top right in Figure 1 has been labeled "pathogenic agent" to signify xenobiotics as well as infectious disease organisms. Excreta were included in this circle since work by Smith and Piper (1975) has clearly demonstrated metabolic ammonia as one cause of pathologic alteration in teleost gill. The concentration of pathogenic agents present in a given site is clearly subject to change relative to the source and extent of pollution or to factors governing density of infectious organisms. If certain factors are present in the host (e.g., genetic susceptibility) and if pathogenic agent(s) are present in sufficient concentration in an altered environment (viz., elevated in tempera-

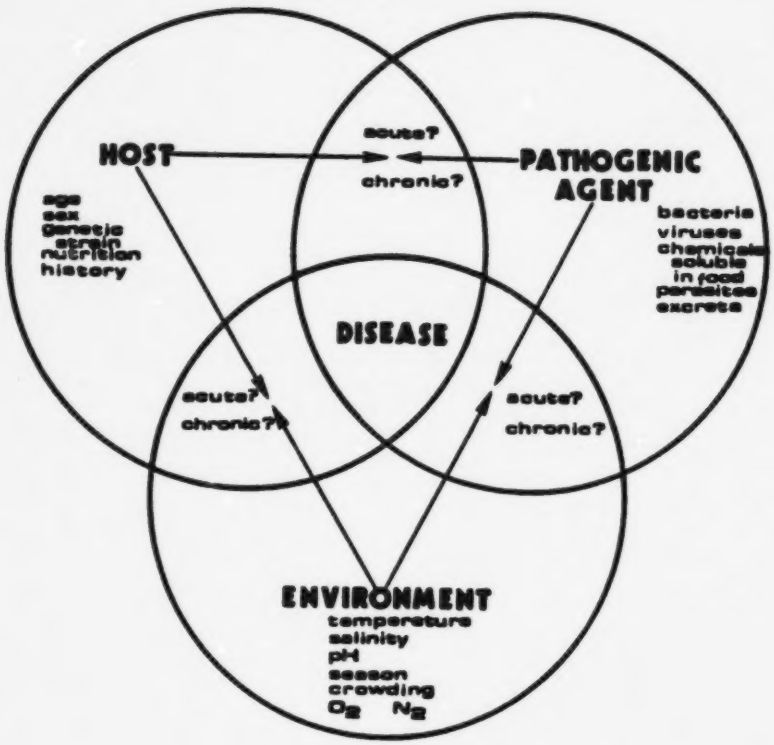

Figure 1. Interactions of environment, host and pathogenic agent(s). (Modified from Snieszko 1974.)

ture (Roberts 1975)) disease may occur (Snieszko 1974). Thus it is important to possess data on host factors, environmental quality and concentration of pathogenic agents.

Examples of important host factors, in addition to those reviewed by Love (1980), are seen in recent studies with the microsomal mixed-function oxidase system of teleost liver. This multienzyme system, induced by specific chemical compounds, plays a central role in xenobiotic biotransformation including activation and detoxification (Lech and Bend 1980; Stegeman 1981; Lech et al. 1982). Stegeman and Chevion (1980) demonstrated sex differences in mixed function oxidase activity in gonadally mature rainbow trout. Stegeman (1981) cites other studies indicating variation in response with age and sex of exposed fish. Pedersen et al. (1976) studied mixed function oxidase activity in six strains of rainbow trout and found differences in constitutive enzyme activity between strains. Following exposure to an inducing agent, differences in response of various genetic strains were evident. Gingerich (1982) reviewed literature on hepatic toxicology of fishes and cited studies indicating that the nutritional status was one of the most important factors in determining the ultimate response of liver tissue to toxicants. In these studies with proven hepatotoxic agents, diet formulations given fish prior to and during exposure to the toxicant greatly affected the extent of toxicity. Following laboratory exposure to certain polycyclic hydrocarbons, polychlorinated biphenyls, polybrominated biphenyls or dioxins, several species of freshwater and marine fish exhibited induction of the hepatic microsomal mixed function oxidase system (see reviews by Lech and Bend 1980; Stegeman 1981). Although environmental pollution has not been conclusively linked to this enzyme response, higher levels of mixed function oxidase components were seen in killifish (Fundulus heteroclitus), from the site of an oil spill 8 years earlier, than were seen in killifish from "nonpolluted" reference marshes (Stegeman 1978). The high level of mixed-function oxidase activity seen in scup (Stenotomous versicolor; see discussion in Stegeman (1981)) and occasionally in sheepshead (Archosargus probatocephalus) (James and Bend 1980) collected from the field, may indicate prior exposure to inducing agents. Since enzyme induction can exert dramatic effects upon the rate of formation of toxic and nontoxic metabolites, carcinogenesis and other endpoints may be affected in populations with prior history of induction (Lech and Bend 1980).

Although the major thrust of laboratory aquatic toxicology studies has centered on host and pathogenic agent(s), recent studies have begun to assess environmental factors mediating response(s) of host to toxicants. Of those factors, temperature has received the most attention. The examples cited here relate to the metabolism of xenobiotics by the microsomal and mixed-function oxidase system. Other examples have been reviewed recently (Love

1980). Under controlled laboratory conditions, temperature has been shown to modify interaction between a xenobiotic compound and the host. James and Bend (1980) collected sheepshead for studies in summer when the water temperature was 26°C and in winter when the water temperature was 14°C. Under summer temperature conditions, exposure to the inducing agent 3-methylcholanthrene caused maximum activity in one microsomal enzyme at 3 days with a return to control values by 14 days after a single dose. Under winter conditions, maximum activity was not seen until 8 days after treatment but elevated activities were still observed at 28 days. The effect of environmental temperature on naphthalene metabolism was studied in the starry flounder (Platichthys stellatus) under controlled laboratory conditions (Varanasi et al. 1981). Flounder, maintained at either 4° or 12°C, were force fed isotopically labeled naphthalene. Analysis of both groups at 24 h post exposure indicated a higher level of metabolites and parent compound in tissues of fish maintained at the lower water temperature. Stegeman (1981) discusses seasonal variations in polycyclic hydrocarbon (benzo-a-pyrene) content in mussels; the highest levels are seen in winter. Since laboratory experimentation has been carried out with oysters (Couch et al. 1979) and shrimp (Couch 1977) the possibility exists for determination of effects of temperature and other environmental factors on toxicity in other marine organisms. Review of the literature on aquatic toxicology serves to underscore further the need for continued effort to define environmental factors and to determine, under controlled laboratory conditions, the extent to which these factors modify the host/pathogenic agent interaction. It is obvious that the work has only begun and that more complex designs for laboratory experiments are necessary to evaluate simultaneously interactions among host, pathogenic agent(s) and environment.

Figure 2 illustrates the role that pathobiology plays in environmental risk assessment. Pathobiologic techniques analyze damage at the various levels of structural organization seen within an organism. Once the extent of the damage has been determined and the number of affected individuals established versus the dose of the toxicant delivered, attempts can be made to predict effects upon populations and species. In addition feral populations can be sampled and alterations described using pathobiologic approaches. The review by Sindermann (1979) cites numerous examples of such studies. When an effect (end point) is seen, retrospective analysis attempts by means of mechanistic connection to link cause and effect (Figure 2). The requirements for construction of a firm association between a disease end point and environmental pollution have been defined in detail (Sindermann 1979). General agreement exists concerning the approach necessary to reach such an important objective. Carefully controlled experimental conditions are necessary to characterize thoroughly the etiology of the process

and to determine the chemicals which can cause the toxic response (Lech and Bend 1980; and Bend and Weber 1980).

Application of Data from Pathobiology to Other Species Including Humans

Since cells and their organelles are the fundamental units in all living species, data at this level of organization, whether obtained in an aquatic or in a terrestrial species, are important since the identical level of organization is shown in humans. Transphyletic similarity exists in enzyme systems which convert pollutants to biologically active (toxic) or inactive metabolites (Bend and Weber 1980). Pathobiology brings together the disciplines of anatomy, biochemistry, pharmacology, physiology and pathology. When coupled with a comparative approach, building upon simpler organization of structure/function and proceeding to more complex systems these tools can lead to an understanding of basic biological mechanisms important in understanding marine pollution effects.

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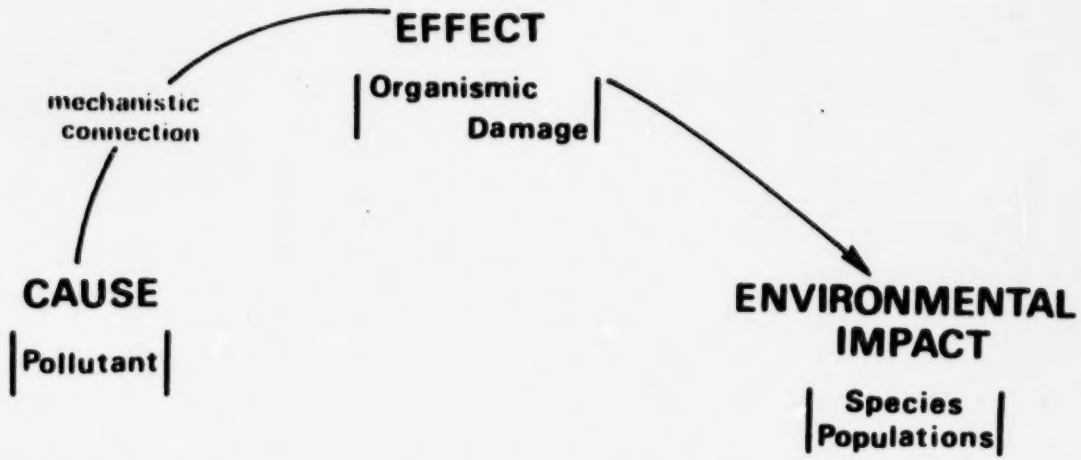

Figure 2. Role of pathobiology in environmental risk assessment. Initial investigation determines effect on organism. Prospective studies based on numbers of individuals affected can be used to predict possible environmental impact. If effect is known, retrospective analysis may lead to cause-effect relationship by means of mechanistic connection. Controlled laboratory tests employing specific chemical toxicants form basis for postulated cause-effect relationship which can then be tested (field validation). (From unpublished work by one of us (J.C.)).

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